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Phylogenetic Relationships Between Mediterranean and Middle-asian Wild Species of the Genus *Hordeum* L. As Revealed by Biochemical and Molecular Markers

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Abstract: The phylogenetic relationships of 60 accessions of the genus *Hordeum* (29 Mediterranean and 20 middle-Asian wild accessions, together with nine American accessions and two of unknown origin), representing together nine species, were investigated by AFLP markers. Three hundred sixty six AFLP fragments were used for studying the molecular genetic diversity among the studied species, 339 out of them were polymorphic. Forty seven protein bands were obtained from the water soluble and the water insoluble seed storage protein by SDS-PAGE electrophoresis of 18 accessions representing nine species (two accessions/species). One band was common to all species and the other 46 bands were polymorphic. The phylogenetic tree deduced from AFLP analysis is concordant to a large extent with that deduced from seed storage protein. Highly significant cophenetic correlation coefficient was obtained between both AFLP (0.96) and seed storage protein (0.89) indicating the reliability of the results. The studied taxa were clustered according to their genome type. All Mediterranean and middle-Asian wild accessions could be integrated into the existing phylogenetic scheme.

Key words: AFLP markers, genetic diversity, *Hordeum* species, phylogeny, storage protein

INTRODUCTION

The genus *Hordeum* contains ca 32 species and ca 45 taxa occurring in temperate areas of Eurasia, North and South Africa and Central and South America. The evolutionary pattern in this genus is complex, including different breeding systems and various forms of polyploidy (Von Bothmer *et al*., 2003). Aberg, 1940 recognized four *Hordeum* sections, *Stenostachys* (Nevski) and *Bulbohordeum* (Nevski) for perennial species and section *Campestria* (Ands) and *Cerealia* (Ands) for annual ones, whereas Nevski (1941) recognized five sections. Love, 1984 separated the genus *Hordeum* into two genera based on genome structure namely *Hordeum sensu stricto*, including both *Hordeum vulgare* and *Hordeum bulbosum* and *Critesion* including the other species. Jaaska (1992) and Jorgensen (1986) studied interspecific relationships in the genus on the basis of the electrophoretic variation of isozymes. Von Bothmer *et al*. (1986, 1987) defined four basic genomes according to the meiotic behavior of different interspecific hybrids and 32 species, genome I for *H vulgare* and *H. bulbosum*, Y for *H. murinum*, X for *H marinum* and H for the remaining *Hordeum* species, *H. halophilum* was added in section *Critesion*. The recent molecular techniques supported mostly the above developed schemes using repetitive DNA sequences, molecular hybridization, RFLP and in situ hybridization (Molnar *et al*., 1989; Molnar and Fedak, 1989; Gonzalez and Ferrer, 1993; Svitashev *et al*., 1994). Blattner (2004) analysed 91 accessions representing all *Hordeum* species. This analysis confirmed the previously formed four clades now named as H, I, X_a and X_u. El Rabey and Al-Maliki, 2011 compared the phylogenetic relationships of the genus *Hordeum* based on 5 AFLP

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primer combinations (E37/M33, E37/M38, E41/M33, E41M40 and E42M38) and ITS sequences of the ribosomal RNA genes. The AFLP markers turned out as a convenient tool to reveal the interspecific genetic diversity in the genus *Hordeum* and the result was concordant with previous studies. The aim of the present study was to reveal the genetic diversity and the phylogenetic relationships between 60 wild accessions of *Hordeum* mostly from Mediterranean and Asian origin which had not been analyzed so far, using AFLP markers and, partly, seed storage protein patterns.

MATERIALS AND METHODS

Plant material: A total of 60 accessions belonging to nine *Hordeum* species either directly sampled from the Egyptian flora or supplied by different gene banks were used for this study as shown in Table 1. The accessions were chosen in order to represent the four main sections according to Von Bothmer *et al*. (1995) and the Old and New World's flora and focused on Mediterranean and middle-Asian origin.

DNA isolation: Plants were grown in the greenhouse. About 20 seeds were sown and young leaves of 3-5 representative plants were collected in sterilized 50 mL polypropylene tubes and lyophilized in a Christ PG 30 freeze-dryer machine. Leaves were ground and kept at -70°C until use. DNA was extracted according to a modified CTAB method (Saghai-Maroof *et al*., 1984).

AFLP markers: AFLP markers were developed according to Vos *et al.* (1995) with following minor modifications. Briefly, the genomic DNA was restricted using EcoRI as rare cutter and MseI as frequent cutter. Double stranded EcoRI and MseI adapters were constructed by MWG-Biotech GmbH, Germany, according to Vos *et al.* (1995) and were ligated to the restricted DNA. The sequences of these adapters are as follows: MseI-adapters: 92A18 (5-GACGATGAGTCCTGAG) and 92A19 (TACTCAGGACTCAT-5), EcoRI-adapters: 91M35 (5-bio-CTCGTAGACTGCGTACC) and 91M36 (CTGACGCATGGTTAA-5). The two primer combinations E40/M38 and E42/M38 were constructed by MWG-Biotech GmbH, (Germany) and used in fingerprinting the studied taxa (Table 2).

Storage protein markers: Both water soluble and water insoluble proteins were extracted from the seeds of 18 accessions (Table 1) that were selected to represent the different four barley genomes. Protein extraction and

Table 1: Barley accessions used for AFLP and storage protein analyses

Code No.	*Hordeum* species	Source	Origin
1*	*H. murinum murinum*	Nordic Gene Bank / 30886.	Unknown
2*	*H. murinum murinum*	Nordic Gene Bank / 30887.	Unknown
3*	*H. vulgare spontaneum*	IPK / 9719.	Libya
4*	*H. vulgare spontaneum*	IPK / 9721.	Libya
5	*H. vulgare spontaneum*	IPK / 9840.	Libya
6	*H. vulgare spontaneum*	IPK / 9823.	Morocco
7	*H. vulgare spontaneum*	IPK / 9826.	Maroc
8	*H. vulgare spontaneum*	SLU / 3139.	Cyprus
9	*H. vulgare spontaneum*	SLU / 3140.	Cyprus
10	*H. vulgare spontaneum*	SLU / 3141.	Cyprus
11	*H. vulgare spontaneum*	SLU / 3142.	Cyprus
12	*H. vulgare spontaneum*	SLU / 3883.	Greece
13	*H. vulgare spontaneum*	SLU / 10288.	Tadzhikistan
14	*H. vulgare spontaneum*	ICARDA / 180007.	Jordan
15	*H. vulgare spontaneum*	ICARDA / 180008.	Jordan
16	*H. vulgare spontaneum*	USDA / 41995.	Israel
17	*H. vulgare spontaneum*	USDA / 406271.	Israel
18	*H. vulgare spontaneum*	ICARDA / 181547.	Libanon,Saida
19	*H. vulgare spontaneum*	ICARDA / 181568.	Libanon, Rachaiya
20	*H. vulgare spontaneum*	USDA / 466024.	Syria
21	*H. vulgare spontaneum*	USDA / 466627.	Iran
22	*H. vulgare spontaneum*	USDA / 219796.	Iran,Khuzestan
23	*H. vulgare spontaneum*	USDA / 253933.	Iraq,Salahidin
24	*H. vulgare spontaneum*	USDA 366431.	Afghanistan, Chekao
25	*H. vulgare. spontaneum*	USDA / 212305	Afghanistan, Mazarisharif
26	*H. vulgare spontaneum*	MPIZ / 2699	Turkmenistan
27	*H. bulbosum*	USDA / 106880.	Turkmenistan
28*	*H. bulbosum*	USDA / 194460.	Israel
29*	*H. bulbosum*	USDA / 205195.	Turkey, Aydin
30	*H. bulbosum*	USDA / 206372.	Cyprus
31	*H. bulbosum*	USDA / 206443.	Turkey, Samsun
32	*H. bulbosum*	USDA 240161.	Israel
33	*H. bulbosum*	USDA / 206890.	Turkey, Eskisehir
34*	*H. bogdanii*	USDA / 269406.	Afghanistan, Kabul
35*	*H. bogdanii*	USDA / 314696.	Kazakhstan
36	*H. bogdanii*	USDA / 440413.	Kazakhstan
37	*H. bogdanii*	USDA / 440414.	Kazakhstan
38	*H. bogdanii*	USDA / 499498.	China
39	*H. bogdanii*	USDA / 499499.	China, Gansu
40	*H. bogdanii*	USDA / 499500.	China
41	*H. bogdanii*	USDA / 499501.	China
42*	*H. brevisubulatum*	USDA / 229448.	Iran
43*	*H. brevisubulatum*	USDA / 531768.	Tajikistan
44	*H. brevisubulatum*	USDA / 531769.	Uzbekistan
45*	*H. marinum*	USDA / 240162.	Libya
46	*H. marinum*	USDA / 200341.	Israel
47*	*H. marinum*	USDA / 223324.	Iran,Khuzestan
48	*H. marinum*	USDA / 283418.	Israel
49	*H. marinum*	USDA / 401364.	Iran
50	*H. marinum*	USDA / 41409.	Israel
51	*H. marinum*	ICARDA / 181278.	Cyprus
52*	*H. chilense*	USDA / 531781.	Argentina,Rio Negro
53*	*H. chilense*	IPK / 972-89.	Chile
54	*H. chilense*	IPK / 987-92.	unknown
55*	*H. pusillum*	USDA / 15654.	USA,Kentucky
56*	*H. pusillum*	USDA / 15663.	USA
57	*H. jubatum*	USDA / 531782.	Mexico, Mexico
58*	*H. jubatum*	USDA 7 566822.	Mexico, Mexico
59*	*H. jubatum*	IPK / 662-84	Bulgaria
60	*H. procerum*	ICARDA /181258.	SouthAmerica

*: Accessions used for storage protein, SDS: PAGE analysis

SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to the method of Laemmli (1970) to study the genetic diversity among the different genomes.

Data analysis: Both AFLP and protein gels were scored as 0/1 for absence/presence of the bands, respectively. Number and percentage of the polymorphic bands were calculated. Similarity coefficient matrices were calculated using Dice similarity algorithm (Dice, 1945) for both markers (AFLP and protein). Phenograms were constructed using the UPGMA method (Unweighted Pair-Group Method with arithmetical algorithms Averages (Sneath and Sokal, 1973) and the correlation cophenetic coefficients were calculated. For the above mentioned analysis, the NTSYS PC2.0 software was used (Rohlf, 1998).

RESULTS

AFLP analysis: Altogether 366 bands were obtained from the AFLP analysis, 339 (93%) out of them were polymorphic (Table 2), 189 bands out of 209 were polymorphic for the first AFLP primer combination (E42-M38) while 150 bands out of 157 were polymorphic for the second primer combination (E40-M38).

The AFLP markers (Fig. 1) and the dendrogram (Fig. 2) separated the barley accessions according to their genome type. All the accessions of *H. vulgare spontaneum* and *H. bulbosum* had the H genome type, whereas the *H. murinum* accessions with genome type X_u were like a subgroup of H. The second group in this cluster with genome type I was also divided into two groups, the first contains all species that have the genome I (i.e., *H. bogdanii, H. brevisbulatum, H. chilense, H. jubatum and H. pusillum*) while the second group contains *H. marinum* which has the X_a genome (Fig. 2). A highly significant correlation cophenetic coefficient was obtained with the dendrogram of the AFLP (r = 0.96) which proved the reliability of the results.

Protein analysis: A high percentage of polymorphism was obtained from the protein analysis, where 46 polymorphic bands out of 47 were scored representing 98% (Table 2, Fig. 3). All the bands obtained from the

Table 2: Sequences and polymorphic bands of AFLP and storage protein markers for the 60 barley accessions

Marker type	Sequence	No. of bands	No. of polymorphic bands	Total (%)
AFLP	E42:5'-GACTGCGTACCAATTCAGT-3' M38:5'-GATGAGTCCTGAGTAAACT-3'	209	189 (90.4%)	93% (339)
	E40:5'-GACTGCGTACCAATTCAGC-3' M38: 5'-GATGAGTCCTGAGTAAACT-3'	157	150 (95.5%)	
Protein	Water soluble	20	20 (100%)	98% (46)
	Water non-soluble	27	26 (96.3%)	

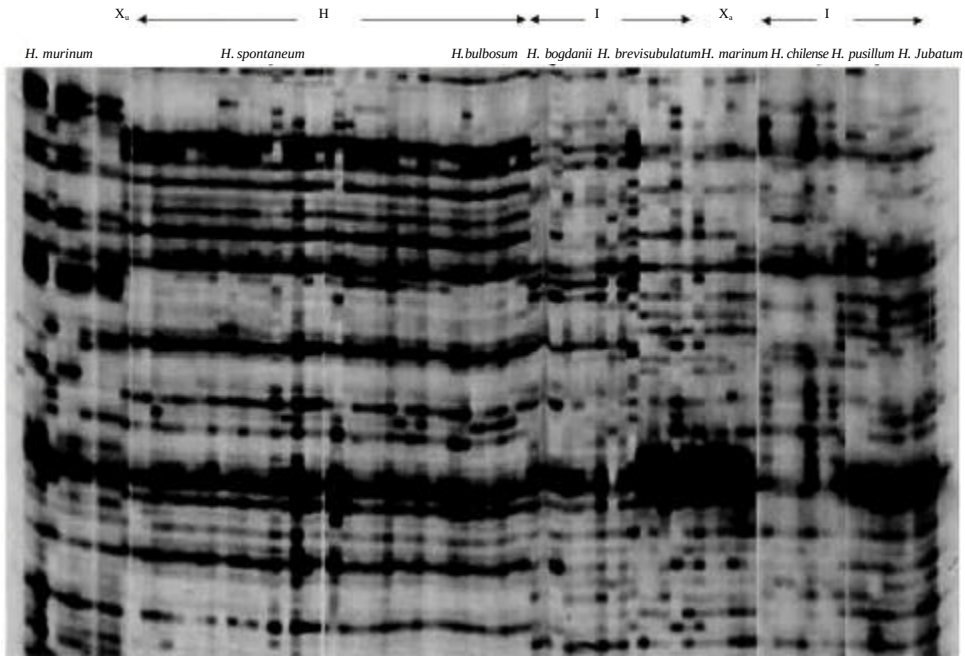

Fig. 1: Part of AFLP banding pattern (E42-M38 primer combination) of accessions of the different genome types

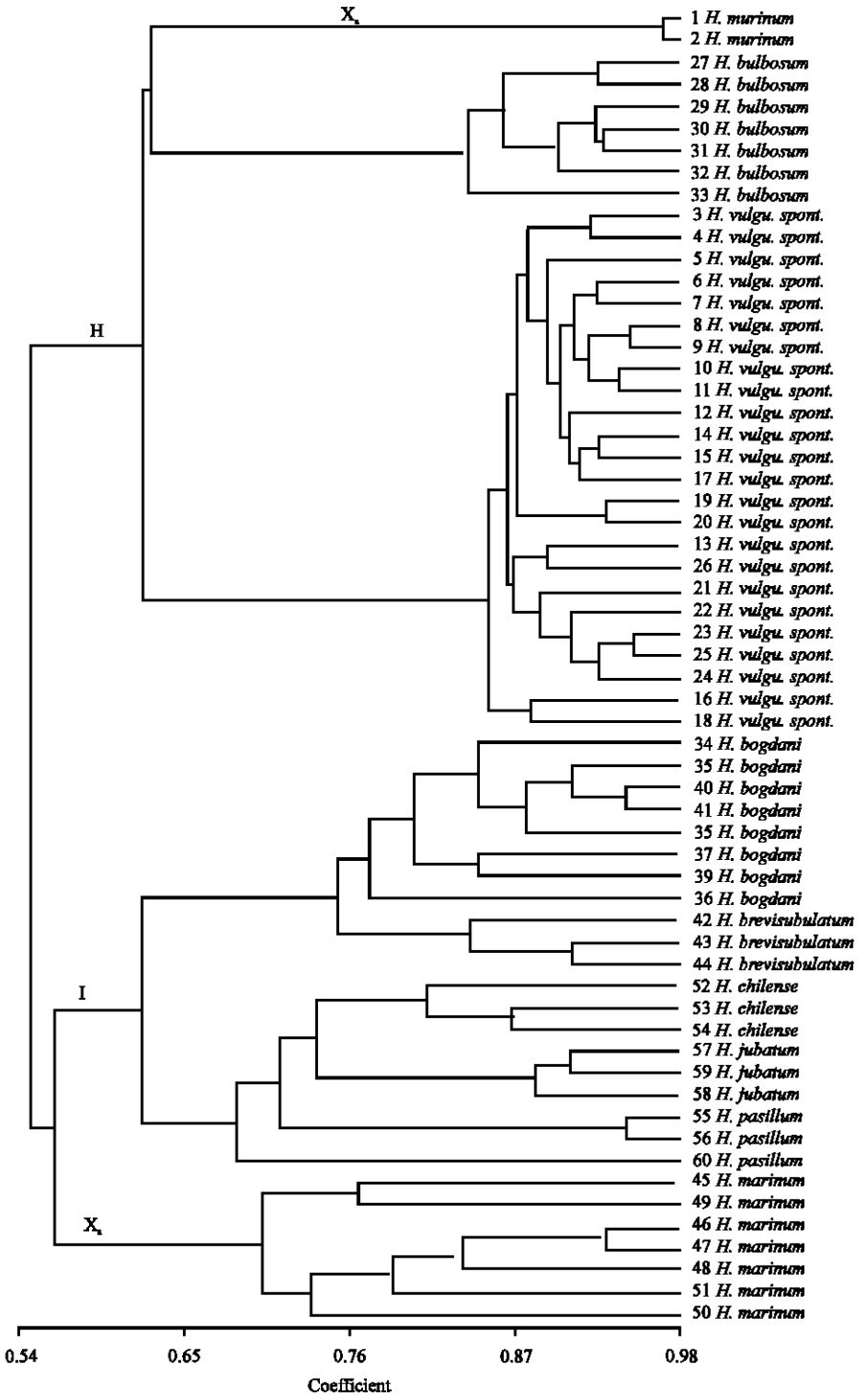

Fig. 2: Dendrogram of the 60 barley accessions using AFLP data based on Dice's similarity coefficient and the UPGMA method

water soluble protein were polymorphic, whereas 26 out of 27 bands were polymorphic for the water insoluble protein (Table 2).

The protein banding pattern of the H-genome accessions was different from the pattern of the I-genome accessions. According to the dendrogram produced from

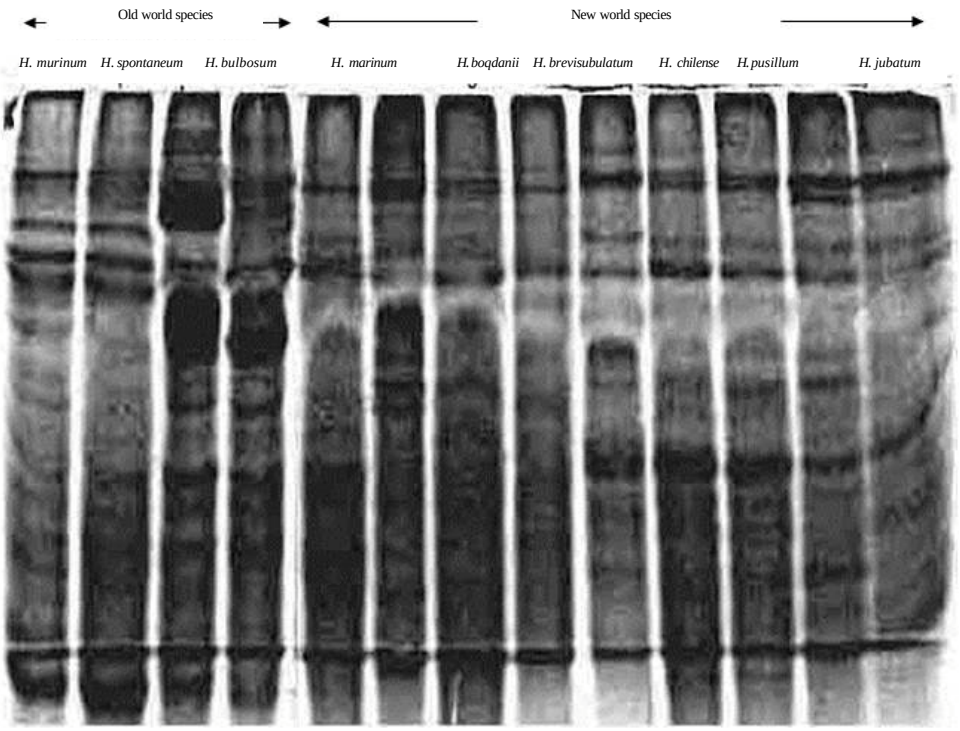

Fig. 3: Part of water insoluble seed storage protein banding pattern of barley accessions representing both Old and New World's species

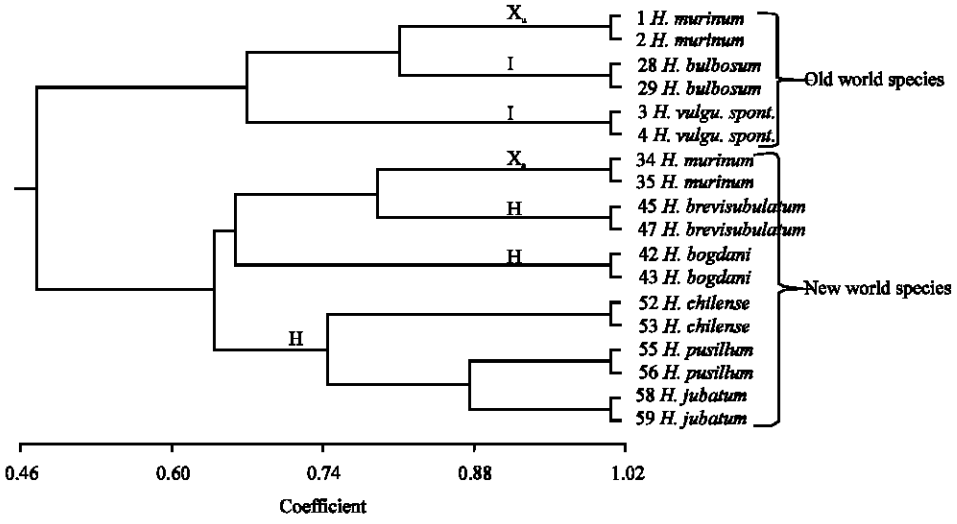

Fig. 4: Dendrogram of 18 barley accessions using both water soluble and water insoluble protein data based on Dice's similarity coefficient and the UPGMA tree building method

the analysis of both water soluble and water insoluble protein, the barley accessions were divided into two clusters similarly to the AFLP results (Fig. 4). The first cluster contains the accessions with genome H (*H. bulbosum* and *H. vulgare spontaneum*) and genome X_u (*H. murinum*) like a subgroup of H. The second cluster consists of the accessions with the genome I (*H. bogdanii, H. brevisbulatum, H. chilense, H. jubatum*

and H. pusillum) and the genome X_a (*H. marinum*) like a subgroup of I (Fig. 4). The reliability of these results was proven by the highly significant correlation of cophenetic coefficient for the protein dendrogram (r = 0.89).

DISCUSSION

The genus *Hordeum* has generally been considered as a well defined and easily recognized monophyletic plant group which is characterized by three one-flowered spikelets at each rachis node, the two lateral ones are either rudimentary or sterile and the central one is fertile in the two-rowed barley, or both of them are fertile in the six-rowed barley. Earlier authors considered all wild species to be fairly closely related to cultivated barley so they thought that all these species constitute genetic resources for breeding purposes, even though rather strong sterility barriers were found to operate (Von Bothmer *et al.*, 1995). Von Bothmer *et al.* (2003) reported that triticeae represents a highly successful evolutionary branch in the grass family (poaceae) and comprises a vast number of species and genera and the numerous wild species are thus potential gene sources for cereal breeding.

Twenty-nine Mediterranean and 20 middle-Asian wild barley accessions were analysed to reveal their location in the barley phylogeny. The phylogenetic analyses based on the 339 AFLP bands and the 46 protein bands divided the studied taxa into two main groups representing the H and the I genome type. It was also noted that accessions of the same species were clustered together. These results are consistent with the recently developed phylogenetic system (reviewed by Blattner, 2009), except that X_u and X_a are more clearly separated there from the H, respectively I group than in our results. The four *Hordeum* genome groups (H, I, Xa, Xu) are monophyletic and contain several allo- and autopolyploidic species. The accessions in our study are mostly diploid, except *H. jubatum* which is tetraploid and *H. procerum* which is hexaploid, Both AFLP data and seed storage protein analyses succeeded in discriminating the accessions according to the genome type and both methods came to the same results. Thus, there are no basic differences between the phylogeny based on AFLP or seed proteins, but there are small differences in the degree of relationships when compared to the scheme by Blattner (2009). The genetic diversity between the studied Mediterranean accessions was only half of that found by Liu *et al.* (2002) with analysis of ten allozymes. Zimmer and Wen (2012) reviewed the current state of low and single-copy nuclear markers that have been applied successfully in plant phylogenetics. They advocate the potential of massively parallel high throughput or Next-Generation Sequencing approaches for future molecular phylogenetic and evolutionary investigations.

The present study focussed especially on barley species and accessions which came from the Mediterranean area. We integrated them into the previously established phylogenetic tree, thus broadening the knowledge of the gene pool which is present in wild barley species from the North African to middle Asian origin.

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REFERENCES

Aberg, E., 1940. The taxonomy and phylogeny of *Hordeum* L. sec. *Cerealia* ands, with special reference to Tibetan barleys. Symbolae Botanicae Upsalienses, 4: 1-156.

Blattner, F.R., 2004. Phylogenetic analysis of *Hordeum* (Poaceae) as inferred by nuclear rDNA ITS sequences. Mol. Phylogenet. Evol., 33: 289-299.

Blattner, F.R., 2009. Progress in phylogenetic analysis and a new infrageneric classification of the barley genus *Hordeum* (Poaceae: Triticeae). Breed. Sci., 59: 471-480.

Dice, L.R., 1945. Measures of the amount of ecological association between species. Ecology, 26: 297-302.

El Rabey, H. and A.L. Al-Maliki, 2011. Application of randomly amplified polymorphic DNA (RAPD) markers and polyphenol oxidases (PPO) genes for distinguishing between the diploid (*Glaucum*) and the tetraploid (*Leporinum*) accessions in *Hordeum murinum* complex. Afr. J. Biotechnol., 10: 13064-13070.

Gonzalez, J.M. and E. Ferrer, 1993. Random amplified polymorphic DNA analysis in *Hordeum* species. Genome, 36: 1029-1031.

Jaaska, V., 1992. Isoenzyme variation in barley (*Hordeum* L.). Aspartate aminotransferase and 6-phosphoglyconate dehydrogenase. Hereditas, 116: 29-35.

Jorgensen, R.B., 1986. Relationships in the barley genus (*Hordeum*): An electrophoretic examination of proteins. Hereditas, 104: 273-291.

Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T_4. Nature, 227: 680-685.

Liu, F., G.L. Sun and B. Salomon, 2002. Characterization of genetic diversity in core collection accessions of wild barley (*Hordeum vulgare* sp. *spontaneum*). Hereditas, 136: 67-73.

Love, A., 1984. Conspectus of the triticeae. Feddes Rep., 95: 425-521.

Molnar, S.J. and G. Fedak, 1989. Polymorphism in ribosomal DNA repeat units of 12 *Hordeum* species. Genome, 32: 1124-1127.

Molnar, S.J., P.K. Gupta, G. Fedak and R. Wheatcroft, 1989. Ribosomal DNA repeat unit polymorphism in 25 *Hordeum* species. Theor. Applied Genet., 78: 387-392.

Nevski, S.A., 1941. Beitrage zur kenntnis der wildwachsenden gersten in zusammenhang mit der Frage den Ursprung von *Hordeum vulgare* L. und *Hordeum distichon* L. zu klaren (Versuch einer Monographie der Gattung *Hordeum* Trudy). Botanski Inst. Akad. Nauk SSSR Ser., 15: 255-264.

Rohlf, F.J., 1998. NTSYS-pc. Numerical Taxonomy and Multivariate Analysis System. Exeter Software, Setauket, New York.

Saghai-Maroof, M.A., K.M. Soliman, R.A. Jorgensen and R.W. Allard, 1984. Ribosomal DNA spacer length polymorphisms in barley: Mendelian inheritance, chromosomal location and population dynamics. Proc. Natl. Acad. Sci., 81: 8014-8019.

Sneath, P.H.A. and R.R. Sokal, 1973. Numerical Taxonomy. WH Freeman and Co., San Francisco, USA.

Svitashev, S., T. Bryngelsson, A. Vershinin, C. Pedersen, T. Sall and R. von Bothmer, 1994. Phylogenetic analysis of the genus *Hordeum* using repetitive DNA sequences. Theor. Applied Genet., 89: 801-810.

Von Bothmer, R., J. Flink and T. Landstrom, 1986. Meiosis in interspecific *Hordeum* hybrids. I. Diploid combinations. Can. J. Genet. Cytol., 28: 525-535.

Von Bothmer, R., J. Flink and T. Landstrom, 1987. Meiosis in interspecific *Hordeum* hybrids. II. Triploid combinations. Evolut. Trends Plants, 1: 41-50.

Von Bothmer, R., K. Sato, T. Komatsuda, S. Yasuda and G. Fischbeck, 2003. The Domestication of Cultivated Barley. In: Diversity in Barley (*Hordeum Vulgare*), Von Bothmer, R., T. van Hintum, H. Knupffer and K. Sato (Eds.). Elsevier Science B.V., Amsterdam, Netherlands, PP: 9-27.

Von Bothmer, R., N. Jacobsen, C. Baden, R.B. Jargensen and I. Linde-Laursen, 1995. An Ecogeographical Study of the Genus Hordeum. 2nd Edn., International Plant Genetic Resources, Rome.

Vos, P., R. Hogers, M. Bleeker, M. Reijans and T.V.D. Lee *et al*., 1995. Homes AFLP: A new technique for DNA fingerprinting. Nucl. Acids Res., 23: 4407-4414.

Zimmer, E.A. and J. Wen, 2012. Using nuclear gene data for plant phylogenetics: Progress and prospects. Mol. Phylogenet. Evol., 65: 774-785.